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THE METABOLISM OF "HISTIDINE-RICH" PROTEIN IN NORMAL AND PSORIATIC KERATINIZATION*

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Historically, the concept of a unique protein synthesized exclusively in the stratum granulosum, originated in our laboratory in 1964 (1) to explain the autoradiographs of Fukuyama, *et al.* (2) which showed the initial incorporation of intraperitoneally injected histidine and glycine in the granular and upper spinous layers of the newborn rat epidermis, whereas methionine, leucine, and phenylalanine first appeared in the lower cells of the epidermis. Support for this hypothesis was provided by Reaven and Cox (3) who showed the presence of copious amounts of histidine in keratohyalin as indicated by the Pauly stain, a relatively specific histochemical reaction for protein-bound histidine.

Subsequently, Hoober and Bernstein (4) isolated a sulfur-poor, "histidine-rich" protein (HRP) from rat epidermis which Gumucio *et al.* (5) then obtained from a tissue preparation composed of stratum corneum and stratum granulosum. This protein has been further purified by Chakrabarti and Bernstein (6) and shown to contain predominantly serine, threonine, arginine, glycine, alanine, aspartic acid (and/or its amide), glutamic acid (and/or its amide), tyrosine and histidine. No sulfur-containing amino acids were found nor did the intraperitoneal injection of cystine-^{35}S result in the labelling of this protein (6). Fukuyama and Epstein (7) studied the localization of six additional tritiated amino acids in the epidermis of the newborn rat and found that arginine and serine were also incorporated initially in the upper viable cellular layers while lysine and valine appeared in the lower layers. Proline and tyrosine were evenly distributed in the basal, spinous and granular layers.

Our laboratory has recently reported preliminary data on the *in vitro* synthesis of HRP in minced epidermis of the newborn rat (8) and on the isolation of keratohyalin protein from the granular layer of the same tissue (9). Using ultrastructural autoradiography, Cox and Reaven (10) and later Fukuyama and Epstein (11) detected an increasing quantity of labelled histidine within keratohyalin. If HRP is a precursor of keratohyalin (11), then the presence or absence of HRP might coincide with the presence or absence of keratohyalin. The data presented in this paper support this prediction. No significant synthesis of HRP was detected in keratohyalin-free psoriatic plaques as compared with that found in the uninvolved epidermis of the psoriatic. A small amount of synthesis occurred in a healing psoriatic plaque.

MATERIAL AND METHODS

Isolation of HRP from necropsy material. Skin specimens obtained at necropsy from various sites including the palm, sole, abdomen and arm, were mechanically defatted and stirred in 1 N NH_4OH for 30 minutes at room temperature. The epidermis was then gently teased from its dermal moorings and pooled. This pooled epidermis was submitted to the published procedure for the isolation of HRP (4) as diagrammed in Fig. 1. Figs. 2 and 3 are histologic pictures of the epidermal material from which the HRP was obtained. The specimens were fixed in 10 percent formalin and stained in the usual way with hematoxylin and eosin (H and E) or by a modification of the Pauly reaction (3). The intense concentration of melanin in the basal zone indicates a separation plane near the dermal-epidermal junction. Amino acid analysis was done automatically on material hydrolyzed in 6 M HCl at 108–110° *in vacuo* for 24 hours by the ion exchange method of Spackman, *et al.* (12).

Intradermal injections and biopsy procedures. Seven microcuries of tritiated L-histidine (4.7 c/mmole) were injected intradermally into each site. At varying intervals, the control injection sites were excised and the epidermis obtained by treatment with 1 N NH_4OH as described above.

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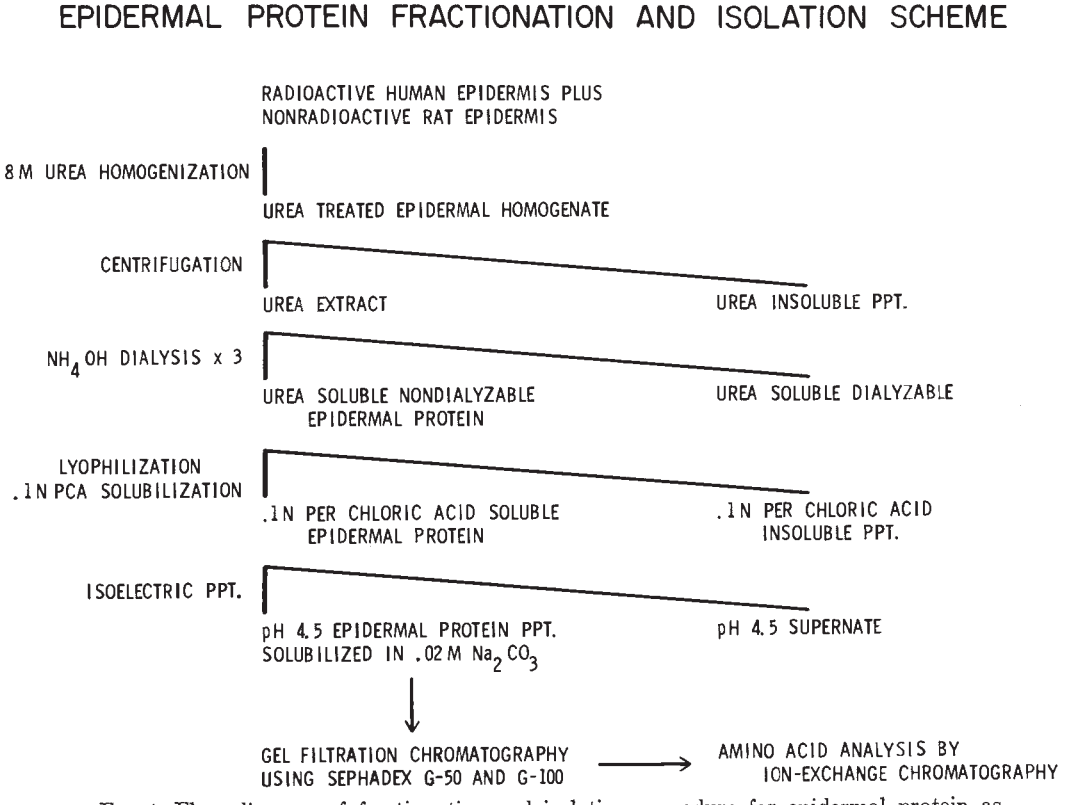

FIG. 1. Flow diagram of fractionation and isolation procedure for epidermal protein as modified from the technique of Hoober and Bernstein (4). PCA = perchloric acid.

Psoriatic and healing psoriatic tissues were obtained by a scalpel incision parallel to the surface of the skin and at a level which allowed punctate, free capillary bleeding in the wake of the surgical slice. The remaining psoriatic dermis was excised and this, plus punch biopsies (4 mm) taken adjacent to the biopsy sites, were processed for H and E staining, the Pauly reaction and autoradiography. The separated epidermal samples and the psoriatic slices were submitted to the isolation procedure for HRP as outlined in Figure 1. Nonradioactive rat epidermis was added to the small biopsy specimens to facilitate isolation of HRP. Autoradiography was done using Eastman Kodak NTB-3 liquid emulsion and an exposure of 4 weeks. Separate sections were stained with H and E and the Pauly reagent. Radioactivity in the protein fractions was detected by liquid scintillation spectrometry as previously reported (4). Protein was measured by the method of Lowry, *et al.* (13).

RESULTS

Isolation of "histidine-rich" protein (HRP) from necropsy skin. The HRP obtained by precipitation at pH 4.5, followed by solubilization in sodium carbonate, was submitted to chromatography on Sephadex G-50 where neither the protein nor the Blue Dextran marker molecules entered the Sephadex matrix, thus being excluded from the column simultaneously. The results of this procedure are given in Figure 4. The protein fraction excluded on Sephadex G-50 was retarded and resolved into two peaks (Fig. 5) when chromatographed on Sephadex G-100. This chromatographic behavior, in conjunction with previous analytical ultracentrifugation of newborn rat HRP, places the molecular weight of human HRP at about 30,000. In tracer studies done in the rat, the first Sephadex G-100 peak contained the HRP. Figure 6 compares the amino acid composition of this peak and the HRP obtained from the epidermis of the newborn rat. The first 9 amino acids are comparable qualitatively, but not quantitatively, in man and the rat. The next 5, with small amounts in man and none in the rat, possibly reflect inclusion of several early fractions of the second Sephadex G-100 peak in the amino acid analysis. Notable by their ab-

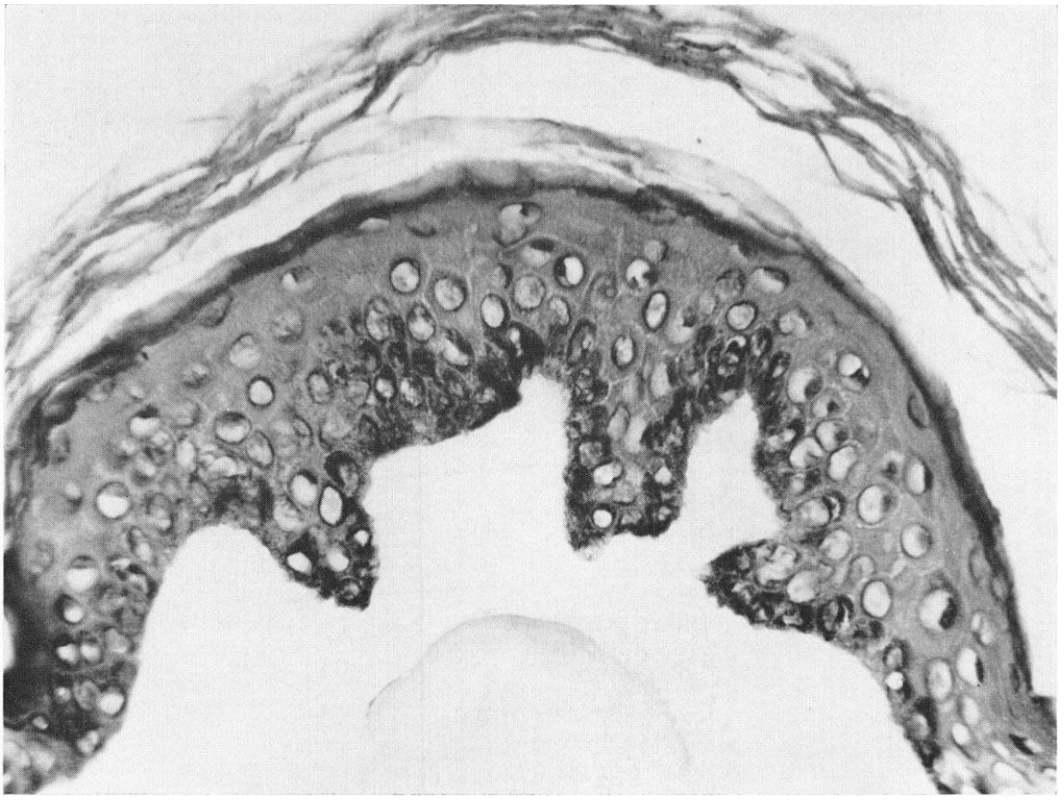

FIG. 2. H and E stained section of epidermis separated in 1 N NH_4OH from necropsy skin. Magnification: 180 ×.

sence are the sulfur-containing amino acids, cystine and methionine.

Tracer experiments. Seven tracer experiments, in three psoriatic volunteers, were carried out to establish concomitance of keratohyalin and HRP. Tritiated histidine was injected intradermally into these sites: psoriatic plaques, a healing psoriatic plaque, and uninvolved control sites, including the palm. At appropriate intervals shown in Table I these sites were excised for biochemical fractionation and autoradiography.

Figure 7 demonstrates that tritiated histidine given by intradermal injection accumulates in normal human stratum granulosum as it does in the stratum granulosum of the rat. Figure 8 shows the coincidence of maximum radioactivity and Pauly positivity in this same layer. Figure 9 shows a control biopsy site. Involution of the area beneath the biopsy site was documented during two months of continuous inpatient observation. Figure 10 is a section of this healing psoriatic tissue where there is the focal presence and absence of keratohyalin. Figure 11 is a section of the psoriatic material used for biochemical fractionation showing the complete absence of keratohyalin.

Table I gives the distribution of radioactivity in the various epidermal protein fractions. The last line of the table represents the control for the psoriatic lesion while the other control relates to the healing psoriatic tissue. Two additional control experiments and one additional experiment with psoriatic tissue were done. The data obtained in these experiments paralleled those shown in the table.

The "pH 4.5" precipitate contains the HRP. As can be seen in Table I, about 20 per cent of the total ^3H in the control epidermis was found in this fraction whereas less than 0.1 per cent appeared here in the case of the psoriatic lesion. Interestingly, 2.5 per cent of the radio-

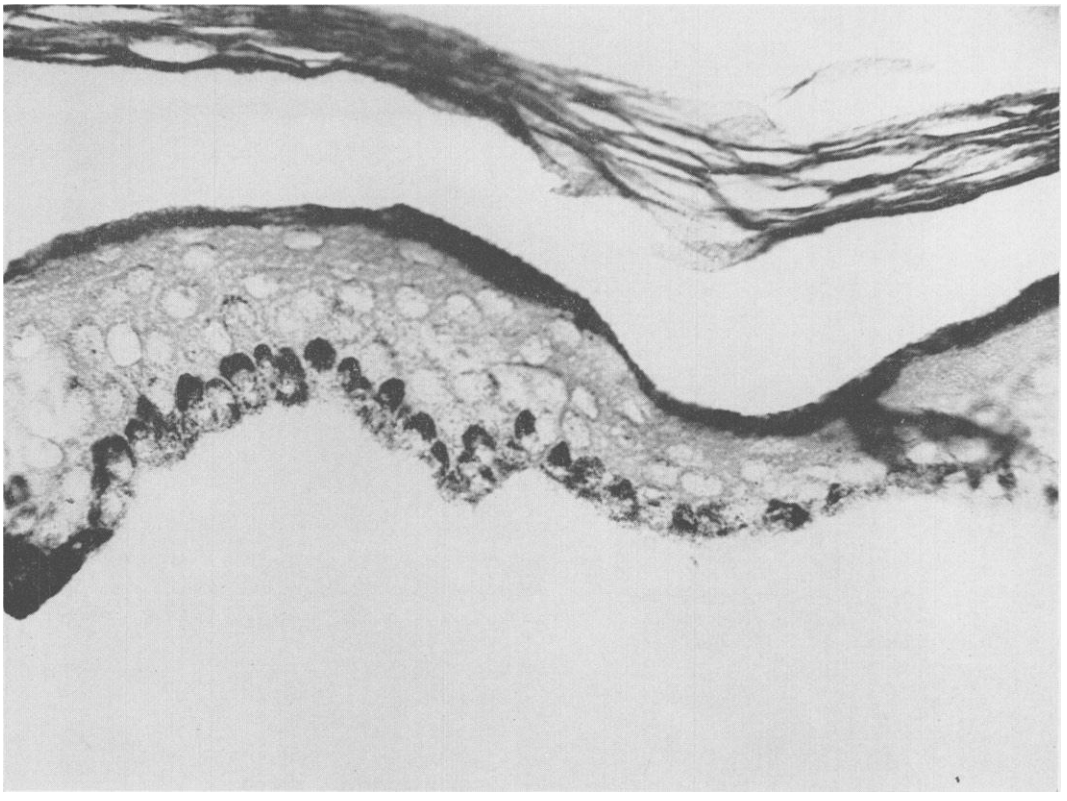

FIG. 3. Pauly stained section of epidermis separated in 1 N NH_4OH from necropsy skin. Magnification: 180 ×.

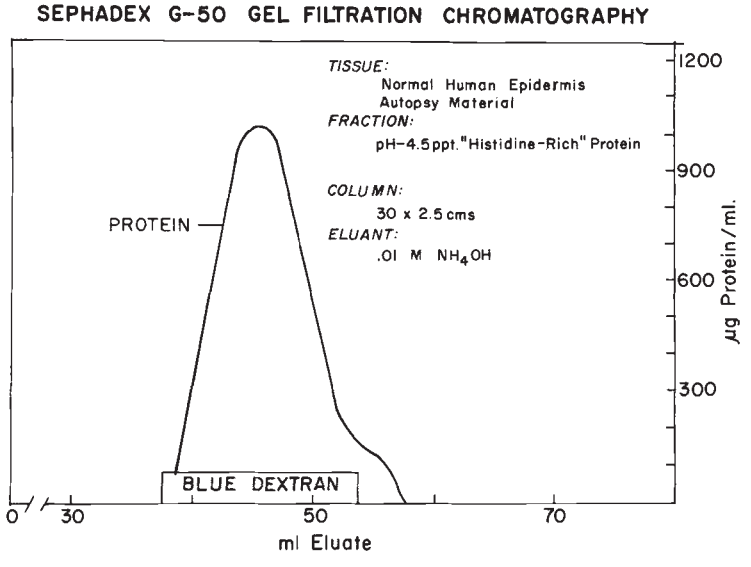

FIG. 4. Elution of the "pH 4.5" precipitated epidermal protein (normal human) from Sephadex G-50.

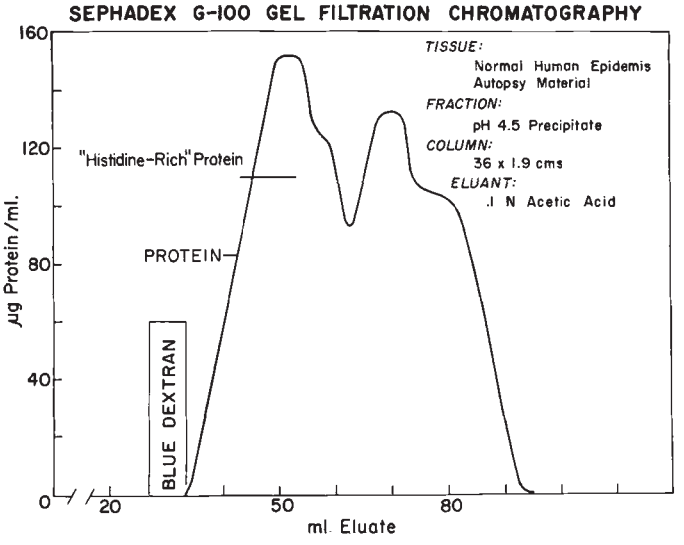

FIG. 5. Elution of "pH 4.5" precipitated epidermal protein (normal human) from Sephadex G-100. The sample chromatographed was material previously excluded from a column of Sephadex G-50.

AMINO ACID COMPOSITION "HISTIDINE-RICH" PROTEIN

AMINO ACID	MAN	NEWBORN RAT
	Residues / 100 Residues	
Threonine	10.7	6.8
Aspartic (NH_2)	15.4	5.6
Glutamic (NH_2)	12.0	14.1
Alanine	10.1	11.8
Tyrosine	4.3	9.4
Glycine	16.8	15.3
Arginine	10.0	9.0
Histidine	8.3	6.9
Serine	6.0	11.5
Valine	1.8	–
Isoleucine	1.2	–
Leucine	1.6	–
Phenylalanine	.6	–
Lysine	1.3	–
Methionine	–	–
1/2 Cystine	–	–

FIG. 6. Comparison of the amino acid composition of HRP isolated from human and newborn rat epidermis. See text for details of analytical method.

TABLE I

Distribution of radioactivity in epidermal protein fractions derived from biopsies taken after intradermal injection of histidine-^3H

HISTIDINE ^3H IN EPIDERMAL PROTEIN FRACTIONS

BIOPSY SPECIMEN	TIME AFTER INJECTION	100% = DPM	UREA EXTRACT	UREA INSOLUBLE PPT.	UREA SOLUBLE NONDIALYZABLE	UREA SOLUBLE DIALYZABLE	PCA† INSOLUBLE PPT.	pH 4.5 PPT.
	Hours	DPM	DPM %	DPM %	DPM %	DPM %	DPM %	DPM %
Psoriasis	1 ½	45,800	99.0	.8	.7	98.0	< .1	< .1
Healing Psoriasis	½	42,000	88.0	12.0	17.0	71.0	7.2	2.5
Control	½	14,200	75.0	25.0	67.0	8.5	32.0	21.0
Control	1 ½	7,340	96.0	3.5	96.0	< .7	21.0	18.0

† = perchloric acid. For details of procedure see text and Fig. 1. DPM = disintegrations per. minute.

FIG. 7. H and E stained autoradiograph of human palm epidermis 1½ hours after intradermal injection of histidine-^3H. Magnification: 540 ×.

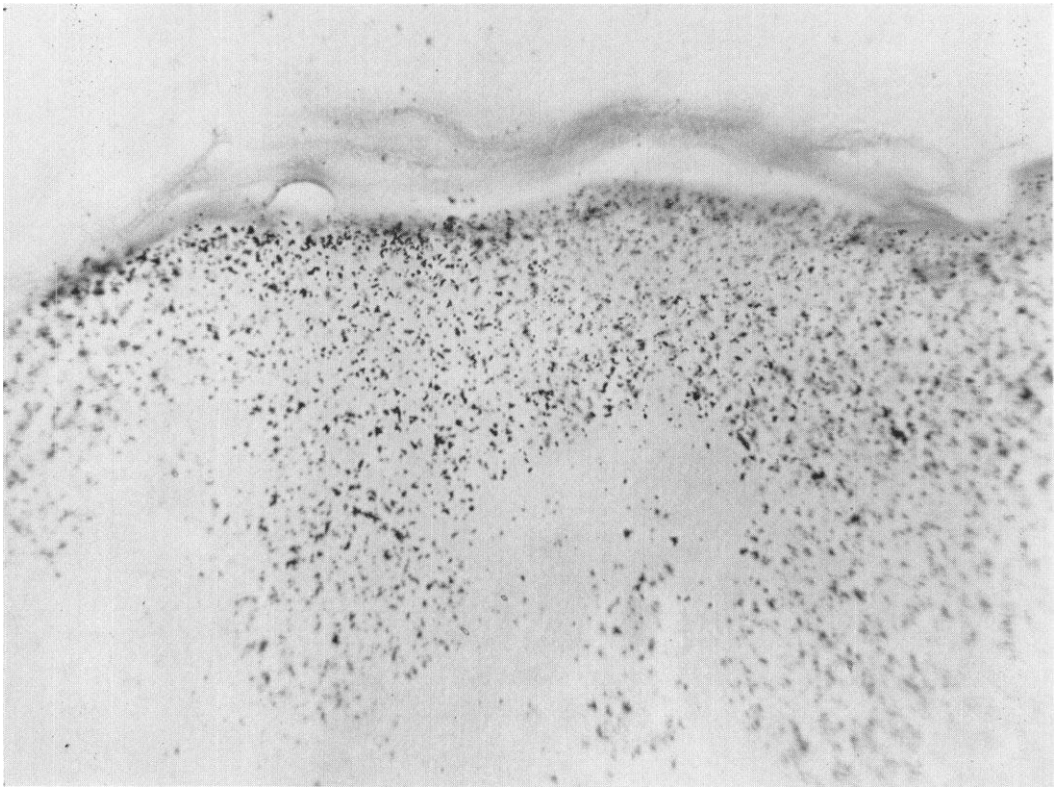

FIG. 8. Pauly stained autoradiograph of normal human epidermis (taken from the site indicated by the arrow in Fig. 9) ½ hour after intradermal injection of histidine-^{3}H. Magnification: 180 ×.

activity appeared in the "pH 4.5" precipitate from the healing lesion in which keratohyalin was reappearing (Fig. 10). The data were similar for the "$HClO_4$-insoluble" fraction. While in the uninvolved epidermis most of the ^{3}H in the urea extract was nondialyzable, nearly all the tritium in the psoriatic urea extract—and to a slightly lesser extent in the healing psoriatic tissue—was dialyzable and therefore in small molecules.

DISCUSSION

The protein obtained in the "pH 4.5" precipitate meets the following established criteria for HRP as isolated from the rat: (a) solubility in 8 M urea, (b) nondialyzability, (c) solubility in 0.1 N $HClO_4$ at room temperature, (d) precipitability at pH 4.5, (e) exclusion on Sephadex G-50, (f) slight retardation on Sephadex G-100, (g) and an unusual amino acid composition showing a high level of histidine.

Very little radioactivity appeared in the "pH 4.5" precipitate in the psoriatic as compared with the control tissue. A higher percentage of the radioactivity appeared in this fraction in the case of healing psoriasis but the control level was not achieved. There are at least four possible explanations for this failure to detect HRP in psoriatic plaques. (a) It is possible that all of the injected radioactive histidine was quickly converted to urocanic acid by histidase. (b) Possibly, as a result of rapid turnover, radioactive HRP was synthesized and degraded before biopsies were taken at 1½ hours. (c) High dermal blood flow may have prevented injected histidine from reaching the potential keratohyalin compartment. (d) Perhaps no HRP synthesis occurs in psoriatic plaques.

A two and one-half fold increase in histidase has been reported (3) in the psoriatic epidermis. However, calculations using this figure would

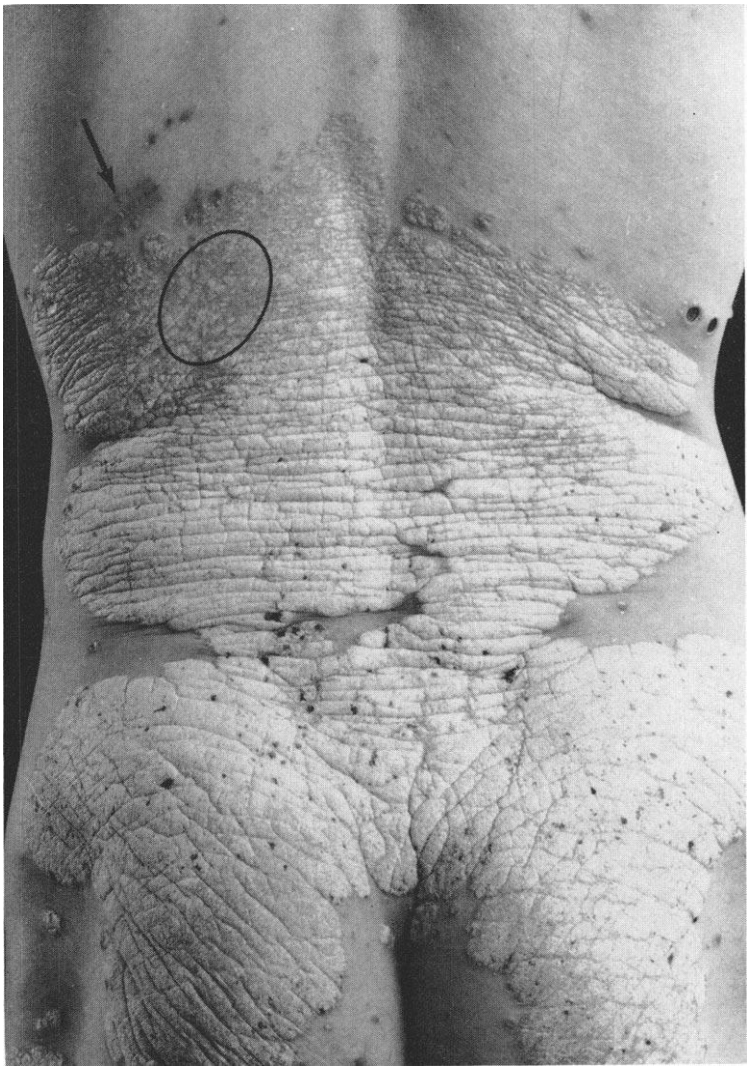

FIG. 9. Photograph made after control (at tip of arrow) and healing (within circle) biopsies were taken. Note the absence of a post-surgical scar in the healing biopsy site.

predict a 60 per cent reduction in the control value rather than the complete absence of radioactivity in the "pH 4.5" precipitate. Furthermore, it has been shown (14) by autoradiography of psoriatic explants exposed to histidine-^{3}H *in vitro*, that there is no accumulation of radioactive histidine in the area of the normal granular zone over a 6-hour period. During this entire period of time, the radioactive precursor was present. The third possible explanation for the failure to detect HRP appears to be excluded by the presence of over 45,000 DPM in the dialyzable fraction of the psoriatic tissue and by the demonstration of fixed radioactivity in the malpighian compartment as shown in the autoradiograph (Fig. 12). It would seem, therefore, that the presence of a biosynthetic block is a reasonable explanation for our failure to detect HRP in psoriatic plaques.

That the "pH 4.5" precipitate is indeed the HRP was indicated by the results of co-chromatography of radioactive human and non-radioactive rat HRP on Sephadex G-50 (Fig. 13). The major peak of radioactivity and the protein peak are superimposable and both are

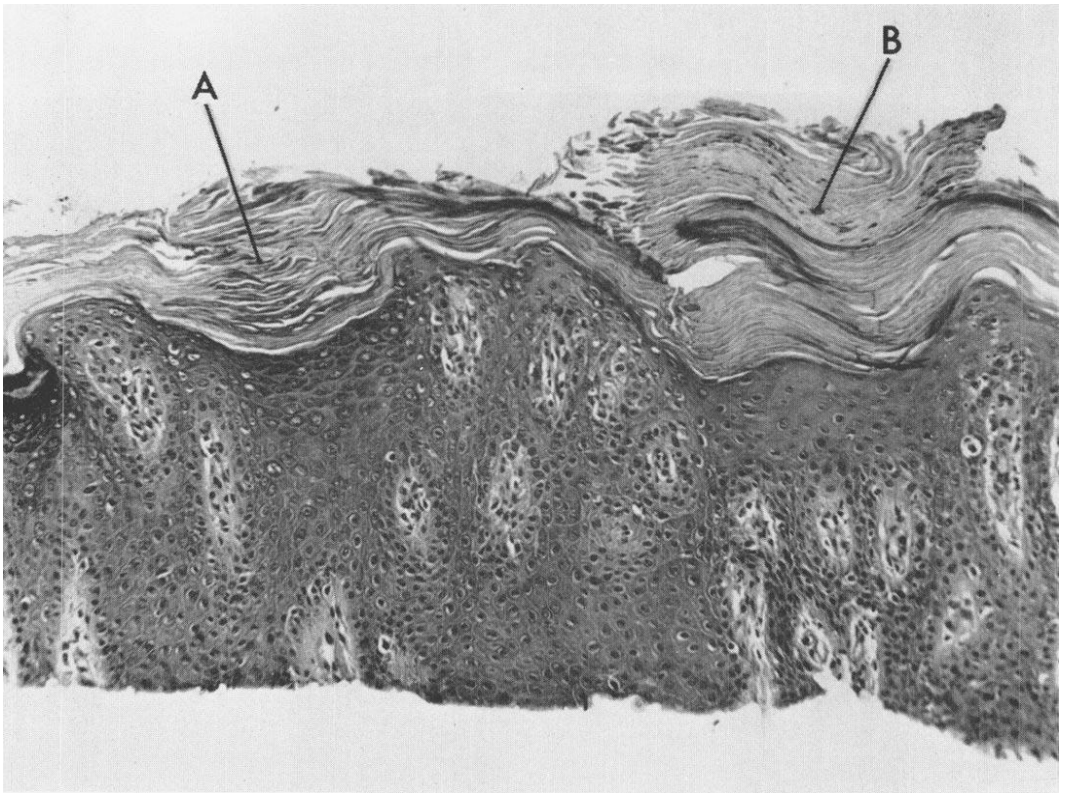

FIG. 10. H and E stained tangential section of the surgical slice through the healing site shown in Fig. 9. *A* indicates orthokeratosis with keratohyalin beneath. *B* indicates parakeratosis with absence of keratohyalin. Magnification: 180 ×.

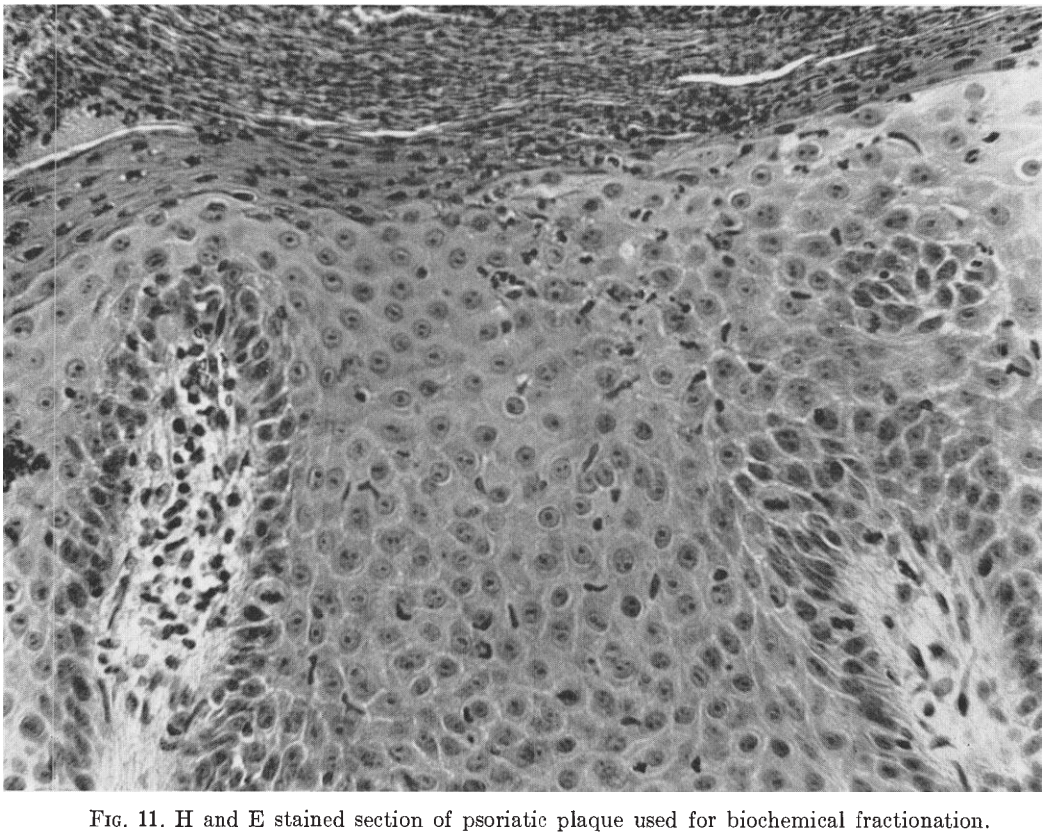

FIG. 11. H and E stained section of psoriatic plaque used for biochemical fractionation.

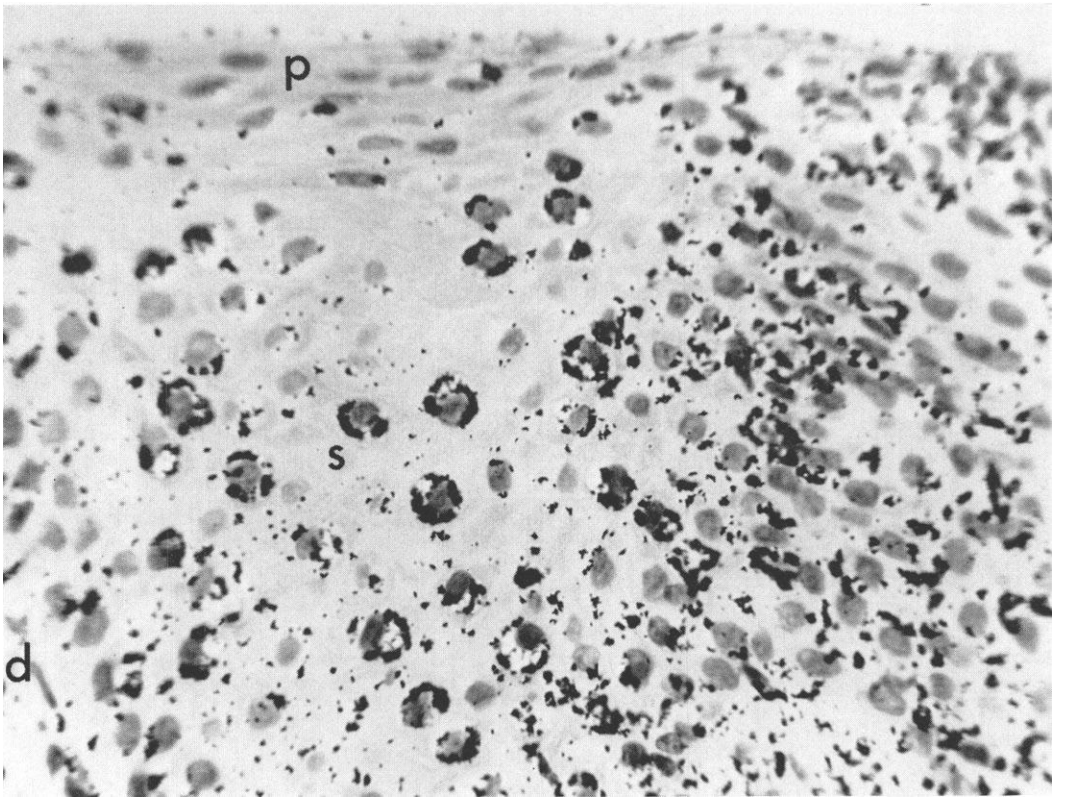

FIG. 12. H and E stained autoradiograph of punch biopsy specimen taken adjacent to the excised psoriatic lesion. Magnification: 540 ×.

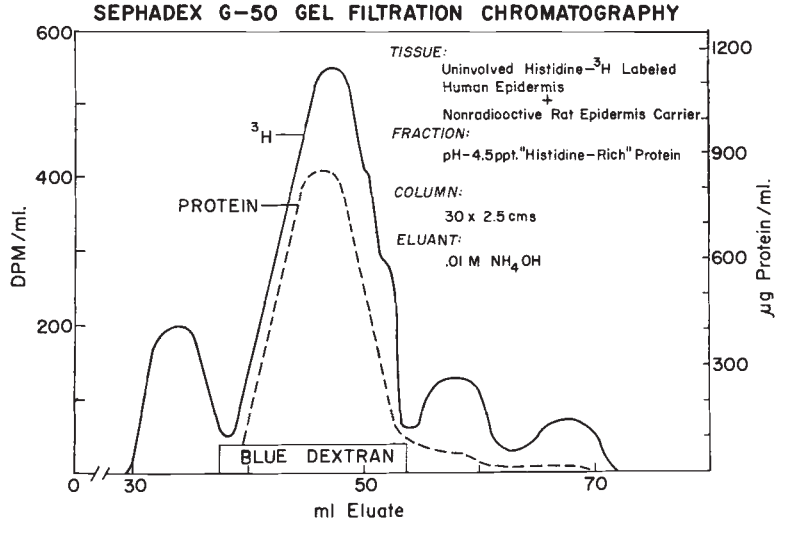

FIG. 13. Elution of "pH 4.5" precipitate from uninvolved human and non-radioactive rat epidermis co-chromatographed on Sephadex G-50.

excluded, with the Blue Dextran marker, exactly as was the human necropsy HRP.

Since the biogenesis of keratohyalin appears to be a genetically programmed event occurring normally in the human about 14 days after the keratinocyte leaves the basal layer, its absence in psoriasis might be a result of the failure to derepress the HRP and keratohyalin cistrons. In molecular biologic parlance, this may represent an aberration in the transcription of DNA into the HRP protein messenger RNA, or a failure in translation involving the absence of the required amino acyl transfer RNA's or the proper ribosomal configurations. Internal or external epigenetic phenomena may explain the fact that involved and uninvolved epidermis can be contiguous. These possibilities are amenable to study.

The relation of such a biosynthetic block to the pathogenesis of psoriasis is unknown.

SUMMARY

The sulfur-free, "histidine-rich" protein has been isolated and characterized in human necropsy epidermis and shown to be similar qualitatively to that isolated from the epidermis of the newborn rat. Using tritiated histidine as a tracer, it was possible to demonstrate the synthesis of radioactive HRP in control, uninvolved epidermis where keratohyalin was present and to show the synthesis of a lesser amount in healing psoriatic lesions where keratohyalin was again appearing. No evidence was obtained for synthesis of HRP in the involved tissue in which no keratohyalin was seen.

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